Genetic Encoding Novel Amino Acids in Embryonic Stem Cells for Molecular Understanding of Differentiation to Dopamine Neurons

Grant Award Details

Genetic Encoding Novel Amino Acids in Embryonic Stem Cells for Molecular Understanding of Differentiation to Dopamine Neurons

Grant Type: New Faculty I

Grant Number: RN1-00577

Project Objective: To develop a strategy to engineer cross-linkable, unnatural amino acids (UAA) into key proteins in mESC and/or hESC, enabling purification of complexes and identification of their components. Use system to study Nurr1 function in neural differentiation.

Investigator:

Name:	Lei Wang
Institution:	Salk Institute for Biological Studies
Type:	PI

Disease Focus: Parkinson's Disease, Neurological Disorders

Award Value: $2,587,742

Status: Closed

Progress Reports

Reporting Period: Year 2

View Report

Reporting Period: Year 3

View Report

Reporting Period: Year 4

View Report

Reporting Period: Year 5 +NCE

View Report

Grant Application Details

Application Title: Genetic Encoding Novel Amino Acids in Embryonic Stem Cells for Molecular Understanding of Differentiation to Dopamine Neurons

Public Abstract: Embryonic stem cells have the capacity to self-renew and differentiate into other cell types. Understanding how this is regulated on the molecular level would enable us to manipulate the process and guide stem cells to generate specific types of cells for safe transplantation. However, complex networks of intracellular cofactors and external signals from the environment all affect the fate of stem cells. Dissecting these molecular interactions in stem cells is a very challenging task and calls for innovative new strategies. We propose to genetically incorporate novel amino acids into proteins directly in stem cells. Through these amino acids we will be able to introduce new chemical or physical properties selectively into target proteins for precise biological study in stem cells.

Nurr1 is a nuclear hormone receptor that has been associated with Parkinson's disease (PD), which occurs when dopamine (DA) neurons begin to malfunction and die. Overexpression of Nurr1 and other proteins can induce the differentiation of neural stem cells and embryonic stem cells to dopamine (DA) neurons. However, these DA neurons did not survive well in a PD mouse model after transplantation. In addition, it is unclear how Nurr1 regulates the differentiation process and what other cofactors are involved. We propose to genetically introduce a novel amino acid that carries a photocrosslinking group into Nurr1 in stem cells. Upon illumination, molecules interacting with Nurr1 will be permanently linked for identification by mass spectrometry. Using this approach, we aim to identify unknown cofactors that regulate Nurr1 function or are controlled by Nurr1, and to map sites on Nurr1 that can bind agonists. The function of identified cofactors in DA neuron specification and maturation will be tested in mouse and human embryonic stem cells. These cofactors will be varied in combination to search for more efficient ways to induce embryonic stem cells to generate a pure population of DA neurons. The generated DA neurons will be evaluated in a mouse model of PD. Additionally, the identification of the agonist binding site on Nurr1 will facilitate future design and optimization of potent drugs.

Statement of Benefit to California: Parkinson's disease (PD) is the second most common human neurodegenerative disorder, and primarily results from the selective and progressive degeneration of ventral midbrain dopamine (DA) neurons. Cell transplantation of DA neurons differentiated from neural stem cells or embryonic stem cells raised great hope for an improved treatment for PD patients. However, DA neurons derived using current protocols do not survive well in mouse PD models, and the details of DA neuron development from stem cells are unclear. Our proposed research will identify unknown cofactors that regulate the differentiation of embryonic stem cells to DA neurons, and determine how agonists activate Nurr1, an essential nuclear hormone receptor for DA neuron specification and maturation. This study may yield new drug targets and inspire novel preventive or therapeutic strategies for PD. These discoveries may be exploited by California's biotech industry and benefit Californians economically. In addition, we will search for more efficient methods to differentiate human embryonic stem cells into DA neurons, and evaluate their therapeutic effects in PD mouse models. Therefore, the proposed research will also directly benefit California residents suffering from PD.